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1581 US

U.S. APPLICATION NO. (If known, see 37 CFR 1.5

09/308295
60/039,227

INTERNATIONAL APPLICATION NO.

PCT/US97/21054

INTERNATIONAL FILING DATE

14 November 1997

PRIORITY DATE CLAIMED

05 December 1996

TITLE OF INVENTION

METHODS FOR DIAGNOSING GLAUCOMA AND DISCOVERING ANTI-GLAUCOMA DRUGS

APPLICANT(S) FOR DO/EO/US

CLARK, Abbot F. and WORDINGER, Robert J.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ has been transmitted by the International Bureau.
 - c. ☒ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. ☒ are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. ☐ have been transmitted by the International Bureau.
 - c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
 - d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

A copy of the International Search Report and Written Opinion

U.S. APPLICATION NO (if known, see 37 CFR 1.5) 60/033,227		INTERNATIONAL APPLICATION NO. PCT/US97/21054		ATTORNEY'S DOCKET NUMBER 1581 US	
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17. <input checked="" type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$1070.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$930.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$790.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$720.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$98.00 ENTER APPROPRIATE BASIC FEE AMOUNT =				CALCULATIONS PTO USE ONLY	
				\$	930.00
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	\$	
Total claims	5 - 20 =	0	x \$22.00	\$	0
Independent claims	4 - 3 =	1	x \$82.00	\$	82.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)				\$	+ \$270.00
TOTAL OF ABOVE CALCULATIONS =				\$	1,012.00
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				\$	0
SUBTOTAL =				\$	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$	0
TOTAL NATIONAL FEE =				\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +				\$	0
TOTAL FEES ENCLOSED =				\$	1,012.00
				Amount to be refunded:	\$
				charged:	\$ 1,012.00

a. ☐ A check in the amount of \$ _____ to cover the above fees is enclosed.

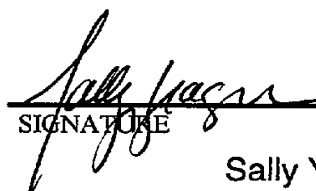
b. ☒ Please charge my Deposit Account No. 01-0682 in the amount of \$ 1,012.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 01-0682. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE
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METHODS FOR DIAGNOSING GLAUCOMA
AND DISCOVERING ANTI-GLAUCOMA DRUGS

Priority is claimed from the provisional application, U.S. Patent Application Serial
No. 60/033227 filed December 5, 1996.

Background of the Invention

Glaucoma is usually diagnosed by monitoring a patient's visual field loss, changes
in the appearance of their optic disc, and their intraocular pressure. Glaucoma is currently
treated using one or more of three strategies to lower the elevated intraocular pressure
associated with the disease: with pharmaceuticals (such as beta-blockers, carbonic
anhydrase inhibitors, and miotics), with laser trabeculoplasty, and/or with glaucoma
filtration surgery. All of these therapies indirectly lower intraocular pressure but do not
address the underlying disease process occurring in the trabecular meshwork. It would be
advantageous to be able to diagnose glaucoma before a patient begins experiencing a loss
in their visual field and deterioration of their optic disc.

There is a large body of evidence suggesting that glucocorticoids are involved in
the generation of ocular hypertension and glaucoma. See Clark, A. F., *Journal of
Glaucoma*, "Steroids, Ocular Hypertension, and Glaucoma," 4:354-369, 1995. Several
investigators have shown that the human trabecular meshwork (TM) contains the classical
glucocorticoid receptor (GR α). See Weinreb, et al., *Invest. Ophthalmol. Vis. Sci.*,
"Detection of Glucocorticoid Receptors in Cultured Human Trabecular Cells," 21:3, 403-
407, 1981, and Hernandez, et al., *Invest. Ophthalmol. Vis. Sci.*, "Glucocorticoid Target
Cells in Human Outflow Pathway: Autopsy and Surgical Specimens," 24:1612-1616,
1983. Recently, the expression of an alternatively spliced form of the human
glucocorticoid receptor (GR β) was discovered in non-ocular tissues and cells. See
Bamberger, et al., *The Journal of Clinical Investigation*, "Glucocorticoid Receptor β , a
Potential Endogenous Inhibitor of Glucocorticoid Action in Humans," 95:2435-2441,
1995, and Oakley, et al., *The Journal of Biological Chemistry*, "The Human
Glucocorticoid Receptor β Isoform," 271:16, 9550-9559, 1996. This alternatively spliced
form of the glucocorticoid receptor (GR) is expressed as a protein which no longer binds
glucocorticoids, but is able to interfere with the activated form of the normal
glucocorticoid receptor and block or alter physiological functions of the glucocorticoid
receptor.

WO 96/14411 discloses a method for diagnosing glaucoma in a patient which comprises determining whether the amount of a trabecular meshwork induced glucocorticoid response protein present in the trabecular meshwork of an eye of a patient exceeds the amount of that trabecular meshwork induced glucocorticoid response protein present in the trabecular meshwork of an eye of an individual who is not suffering from glaucoma, wherein the detection of an excessive amount of the trabecular meshwork induced glucocorticoid response protein is indicative of Glaucoma.

Summary of the Invention

The present invention is directed to methods for diagnosing glaucoma by testing a person for aberrant GR β expression. Also set forth are methods for screening for therapeutic agents useful for treating glaucoma.

Description of Preferred Embodiments

Surprisingly, it has been found that cultured human trabecular meshwork cell lines derived from glaucomatous donors express mRNA for both an alternate splice form of the human glucocorticoid receptor (GR β), as well as the normal glucocorticoid receptor (GR α), whereas normal TM cell lines only express mRNA for GR α . It is believed that the elevated intraocular pressure associated with primary open-angle glaucoma may be due to the aberrant expression of GR β in the trabecular meshwork. Therefore, determining that an individual abnormally expresses GR β in their trabecular meshwork or other tissues can lead to a diagnosis of glaucoma. Also, this discovery can be used to determine whether agents have therapeutic value in treating glaucoma by determining whether they interact with GR β or alter the expression of GR β . This can be done using ligand binding assays or GR β functional assays.

Diagnosing aberrant GR β expression or defects in the GR gene which encodes GR β can be done by using procedures well known to those skilled in the art. See Caskey, C. T., *J.A.M.A.*, "Molecular Medicine. A Spin-off From the Helix," 269:15, 1986-1992, 1993. For example, subjects could be screened for the presence of a genetic defect in GR β by analyzing the DNA derived from peripheral blood leukocytes. Types of DNA analyses could include, but would not be limited to: restriction fragment length polymorphisms (RFLP), single-stranded conformation polymorphisms (SSCP), polymerase chain reaction (PCR), denaturing gradient gels, allele specific oligonucleotide ligation assay, and allele specific hybridization assay. In addition, trabecular meshwork, or other relevant cells from subjects could be analyzed for GR β expression by a number of techniques such as reverse-transcription polymerase chain reaction (RT-PCR), immunoassays, GR functional assays, etc.

We Claim:

1. A method for diagnosing glaucoma which comprises detecting aberrant alternate splice form of the human glucocorticoid receptor (GR β) expression or defects in a GR gene which encodes GR β .
2. The method of Claim 1 wherein GR gene defects are detected by a method selected from the group of assays consisting of: restriction fragment length polymorphism (RFLP), single-stranded conformation polymorphism (SSCP), polymerase chain reaction (PCR), denaturing gradient gel, allele specific oligonucleotide ligation, and allele specific hybridization.
3. A method for diagnosing glaucoma, which comprises detecting genetic changes in the GR gene leading to altered GR β expression.
4. A method for diagnosing glaucoma, which comprises detecting genetic changes outside the GR gene which lead to altered GR β expression.
5. A method for determining whether an agent is useful for treating glaucoma by determining whether it interacts with GR β or alters the expression of GR β .

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am an original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHODS FOR DIAGNOSING GLAUCOMA AND DISCOVERING ANTI-GLAUCOMA DRUGS

(Attorney Docket No. 1581Pr) the specification of which (check one)

- ☐ is attached hereto.
☒ was filed by an authorized person on my behalf on December 5, 1996, as
Application Serial No. 60/033,227

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint James A. Arno, Reg. No. 26,145; Gregg C. Brown, Reg. No. 30,613; Sally Yeager, Reg. No. 32,757; Barry L. Copeland, Reg. No. 34,801; Jeffrey S. Schira, Reg. No. 34,922; Patrick M. Ryan, Reg. No. 36,263; and Michael C. Mayo, Reg. No. 38,545 of Alcon Laboratories, Inc., 6201 South Freeway, Fort Worth, TX 76134, and Robert L. Price, Reg. No. 22,685 of Lowe, Price, LeBlanc, and Becker, my attorneys, with full power of substitution and revocation, to prosecute this application and to transact all business in the United States Patent and Trademark Office connected therewith.

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